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(54) Biosensitive element for surface plasmon resonance measurements, and its method of manufacture

(57) An objective of the present invention is to provide a measuring chip for a surface plasmon resonance sensor that can detect a small amount of target substances in high sensitivity. The present invention provides a measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a biologically active substance immobilized on the surface of said plasma polymerization layer.

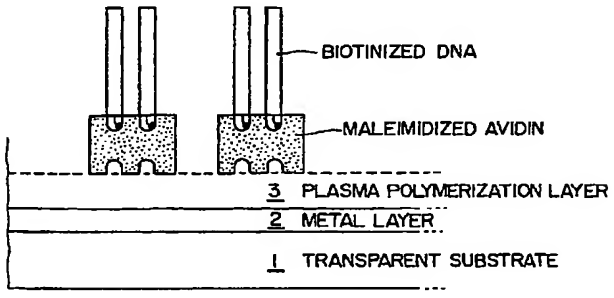

FIG. 1

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] The present invention relates to a surface plasmon resonance biosensor, specifically, a measuring chip for the same and a method for producing the measurement chip.

Background Art

[0002] A number of methods using immunological reactions are used in clinical tests for detecting target substances. Conventional methods are known to be intricate and require labeling substances. Thus, immunological sensors using a surface plasmon resonance biosensor (SPR) is being used, in which no labeling substance is required and a ligand can be detected with high sensitivity. This surface plasmon resonance biosensor is based on the phenomenon that the intensity of a monochromatic light reflected from the interface between an optically transparent substance such as glass and a metal thin-film layer is dependent on the refractive index of a sample placed on the reflecting side of the metal. Accordingly, a sample can be analyzed by measuring the intensity of the reflected monochromatic light.

[0003] An optical part of a measuring cell for this surface plasmon resonance (surface plasmon resonance biosensor) has a structure shown in Figure 2. Namely, porous material 5 is formed on metal thin-film 2 formed on glass substrate 1, and physiologically active substance 4, such as an enzyme or antibody, is retained or immobilized on the surface or inside of porous material 5. Examples of porous material 5 to be used include weaved, knitted or non-woven cloths made of synthetic fibers, natural fibers, inorganic fibers or the like, and porous inorganic or organic materials (see Japanese Patent Laid-open No. 164195/1991). Furthermore, carboxymethyl dextran is used as a porous material in a commercial product (BIAcore 2000, Pharmacia Biosensor).

[0004] However, physiologically active substance 4 just exists on the surface of porous material 5 and interacts with target substances.

[0005] LB (Langmuir-Blodgett) method is occasionally used to immobilize physiologically active substance 4 on metal thin-film 2 (see Japanese Patent Laid-open No. 288672/1993). However, this method has a disadvantage in that LB membrane binds poorly to a metal thin-film and peels off together with the physiologically active substance.

[0006] Furthermore, Japanese Patent Laid-open No. 264843/1997 discloses measuring cells for a surface plasmon resonance biosensor.

SUMMARY OF THE INVENTION

[0007] The present inventors have now found that sensitivity of a measuring chip for a surface plasmon resonance sensor is extremely improved when only a small amount of a physiologically active substance is immobilized on a specific plasma polymerization layer.

[0008] An objective of the present invention is to provide a measuring chip for a surface plasmon resonance sensor that can detect a small amount of target substances in high sensitivity.

[0009] Another objective of the present invention is to provide a measuring cell for a surface plasmon resonance sensor that can detect a small amount of target substances in high sensitivity.

[0010] Further objective of the present invention is to provide a method for producing said measuring chip.

[0011] The present invention provides a measuring chip for a surface plasmon resonance sensor comprising a metal layer and one or more plasma polymerization layers formed on said metal layer.

[0012] The present invention also provides a measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a biologically active substance immobilized on the surface of said plasma polymerization layer.

[0013] The present invention also provides a measuring cell for a surface plasmon resonance sensor comprising said measuring chip.

[0014] The present invention also provides a method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a biologically active substance on the surface of said plasma polymerization layer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015]

Figure 1 is a schematic sectional view of one embodiment of the measuring chip for a surface plasmon resonance sensor according to the present invention.
Figure 2 is a schematic sectional view of an optical part of a measuring chip for a conventional surface plasmon resonance biosensor. 1: Transparent substrate; 2: Metal thin-film; 3: Plasma polymerization layer; 4: Physiologically active substance; 5: Porous material.
Figures 3 (a) and (b) each show a schematic sectional view of an optical part of a measuring chip for a surface plasmon resonance biosensor. (a) shows immobilization of an Fab fragment of an antibody. (b) shows immobilization of an $F(ab')_2$ fragment of an antibody. 1: Transparent substrate; 2: Metal thin-film; 3: Plasma polymerization layer; 4: Physiologically active substance.
Figure 4 is a schematic sectional view of an optical part of a measuring chip for a surface plasmon resonance biosensor.
Figure 5 illustrates a surface plasmon resonance biosensor. 7: Cartridge block; 8: Light source; 9: Detector; 10: Measuring chip; 71: Measuring cell; 72, 73: Flow routes; 80: Incident light; 90: Reflecting light.
Figure 6 illustrates a reflected light intensity curve before and after plasma polymerization membrane formation.
Figure 7 illustrates a schematic view showing the apparatus used in Example 1.
Figure 8 shows the relationship between the concentration of the complementary DNA and RU in Example 1.
Figure 9 shows the relationship between the concentration of the complementary DNA and RU in Example 2.
Figure 10 shows the relationship between the concentration of the complementary DNA and RU in Example 3.
Figure 11 shows the relationship between the concentration of the complementary DNA and RU in Example 4.
Figure 12 shows the relationship between the concentration of the HSA antigen and RU in Example 5.
Figure 13 shows the relationship between the concentration of the BSA antigen and RU in Example 6.
Figure 14 shows the relationship between the concentration of the sugar and RU in Example 7.
Figure 15 shows the relationship between the concentration of the BSA antigen and RU in Example 8.
Figure 16 shows the relationship between the concentration of the BSA antigen and RU in Example 9.
Figure 17 shows the relationship between the concentration of the BSA antigen and RU in Example 10.
Figure 18 shows the relationship between the concentration of the BSA antigen and RU in Example 11.
Figure 19 shows the relationship between the concentration of the HSA antigen and RU in Example 12.
Figure 20 shows the relationship between the concentration of the HSA antigen and RU in Example 13.
Figure 21 shows the relationship between the concentration of the HSA antigen and RU in Example 14.
Figure 22 shows the relationship between the concentration of the HSA antigen and RU in Example 15.
Figure 23 shows the relationship between the concentration of the HSA antigen and RU in Example 16.
Figure 24 shows the relationship between the concentration of the complementary DNA and RU in Example 17.
Figure 25 shows the relationship between the concentration of the HSA antigen and RU in Example 18.
Figure 26 shows the relationship between the concentration of skatole and RU in Example 19.
Figure 27 shows the relationship between the concentration of the HSA antigen and RU in Example 20.
Figure 28 shows the relationship between the concentration of the HSA antigen and RU in Example 21.

DETAILED DESCRIPTION OF THE INVENTION

[0016] The measuring chip for a surface plasmon resonance sensor ("measuring chip") may have optically transparent substrate (transparent substrate) 1, metal thin-film 2 formed on transparent substrate 1, plasma polymerization layer 3 formed on metal thin-film 2, and physiologically active substance 4 immobilized on the surface of plasma polymerization layer 3 as shown in Figure 1.

[0017] Transparent substrate 1 can be any substrate customarily used in a measuring chip for a surface plasmon resonance sensor. Generally, substrates made of materials that are transparent to a laser beam such as glass can be used. The thickness of the substrate can be about 0.1 to 5 mm.

[0018] Metal thin-film 2 is not particularly restricted, provided it can induce surface plasmon resonance. Examples of the metal to be used for metal thin-film 2 include gold, silver and platinum. They can be used alone or in combination. Furthermore, for better adhesion to transparent substrate 1, an auxiliary layer made of chrome or the like may be set between transparent substrate 1 and the layer made of gold, silver or the like.

[0019] The thickness of metal thin-film 2 is preferably 100 to 2000 angstroms, most preferably 100 to 500 angstroms. If the thickness exceeds 3,000 angstroms, surface plasmon phenomena of the medium cannot be sufficiently detected. Furthermore, when an auxiliary layer made of chrome or the like is formed, the thickness of the auxiliary layer is preferably 30 to 50 angstroms.

[0020] Plasma polymerization layer 3 can be formed by plasma polymerization of a monomer material for three-dimensional cross-linking. A monomer material to be used in the present invention can be any material that can immobilize a physiologically active substance by plasma polymerization.

[0021] Examples of a monomer material for a plasma polymerization layer include compounds of formula (I):

$$CH_3\text{-}(CH_2)_n\text{-}NH_2 \text{ (wherein n is an integer from 1 to 6)} \quad (I)$$

and compounds of formula (II):

$$NH_2\text{-}(CH_2)_n\text{-}NH_2 \text{ (wherein n is an integer from 1 to 6)} \quad (II)$$

and compounds which comprise carbon (C), hydrogen (H) and nitrogen (N) and have double bonds or triple bonds, such as acetonitrile, vinylamine and pyridine.

[0022] Furthermore, when a cross-linking reagent or a condensation reagent is used as a linking layer, a compound further containing sulfur (S), oxygen (O) or silicon (Si) can be used as a monomer material. Generally, a compound appropriately containing any two or more elements selected from carbon (C), hydrogen (H), nitrogen (N), sulfur (S), oxygen (O) and silicon (Si) can be used. In addition, a halogen gas or a rare gas can be used as a monomer material.

[0023] In the present invention, a compound containing nitrogen can be used as a monomer material. Examples of the compound containing nitrogen include nitrogen N_2; ammonium; hydrazine; pyridine; compounds of formulae (I) and (II) such as ethylenediamine $NH_2(CH_2)_2NH_2$, hexamethylenediamine $NH_2(CH_2)_6NH_2$, n-propylamine $CH_3(CH_2)_2NH_2$ and monoethylamine $CH_3(CH_2)NH$; compounds of formula $(CH_3)_3(CH_2)_nN$ (n=0 to 17) such as triethylamine $(C_2H_5)_3N$; compounds of formula $(CH_3)_2(CH_2)_nNH$ (n=0 to 17) such as diethylamine $(C_2H_5)_2NH$; compounds of formula $CH_2=CH(CH_2)_nNH_2$ (n=0 to 17) such as allylamine $CH_2=CHCH_2NH_2$; compounds of formula $CH_3(CH_2)_nCN$ (n=0 to 17) such as acetonitrile CH_3CN; compounds of formula $CH_3(CH_2)_nCN$; propargylamine $CHCCH_2NH_2$; compounds of formula CHC $(CH_2)_nNH_2$; acrylamide; aniline; acrylonitrile; 1,2,4-triazole; and 5-amino-1H-tetrazole.

[0024] Further examples of of the compound containing nitrogen include the following:

$RaNRb_2$:

Ra is H or $CH_3(CH_2)_n$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group, and
Rb is H or $CH_3(CH_2)_n$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group;

RaNRc:

Rc is H or $CH_3(CH_2)_nCH$ (n=0 to 17), or CH_2,
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group;

RdN:

Rd is $CH_3(CH_2)_nC$ (n=0 to 17) or CH,
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group;

$ReNRfNRg_2$:

Re is H or $CH_3(CH_2)_n$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group,
Rf is $(CH_2)_n$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group,
Rg is H or $CH_3(CH_2)_n$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or

cyclized group; RhNRiNRj

Rh is H or $CH_3(CH_2)_n$ (n=0 to 17) or $CH_3(CH_2)_n$ CH (n=0 to 17) or $CH)_2$,
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group,

Ri is $(CH_2)_n$ (n=0 to 17) or CH $(CH_2)_n$ CH (n=0 to 17),
and includes a group having a double bond a triple bond or both in the chain, and further a branched or cyclized group,

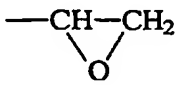
Rj is H or $CH_3(CH_2)_n$ CH (n=0 to 17) or $CH_3(CH_2)_n$CH (n=0 to 17) or CH_2 or $CH_3(CH_2)_n$ C (n=0 to 17) or CH,
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group;

NRkN:

Rk is $C(CH_2)_nC$ (n=0 to 17),
and includes a group having a double bond or a triple bond or both in the chain, and further a branched or cyclized group.

[0025] In the present invention, a compound containing sulfur can be used as a monomer material. Examples of the compound containing sulfur include hydrogen sulfide; carbon disulfide thiophene; compounds of formula $CH_3S(CH_2)_nCH_3$ (n=0 to 17) such as dimethyl sulfide $(CH_3)_2S$; compounds of formula $CH_3(CH_2)_nSS(CH_2)_mCH_3$ (n=0 to 17, m=0 to 17) such as methyl disulfide CH_3SSCH_3; compounds of formula $CH_3(CH_2)_nSH$ (n=0 to 17) such as ethanethiol CH_3CH_2SH; compounds of formula $SH(CH_2)_nSH$ (n=1 to 17) such as ethanedithiol $SH(CH_2)_2SH$; mercaptoethanol; and dithreitol.

[0026] Furthermore, compounds having one of or two or more of groups including -COOH, -CHO, -SH, $-NH_2$, -OH, =NH, $CONH_2$, -NCO, $-CH=CH_2$, =C=O and



can be used as a monomer material. Examples of such compounds include cysteine, glutathione, formyl succinate, aminobenzoate, aminohexanate, mercaptobenzoate, and compounds having $-C{\equiv}CCH_2OH$.

[0027] In the present invention, a compound containing a halogen can be used as a monomer material. Examples of the compound containing a halogen for a plasma polymerization layer include tetrafluoroethylene, chlorobenzene, hexachlorobenzene, hexafluorobenzene, and vinyl fluoride.

[0028] In the present invention, an organic metal compound can be used as a monomer material. Examples of an organic metal compound for the plasma polymerization layer include an organic silicon compounds such as tetramethylsilane, tetramethyldisiloxane, hexamethyldisiloxane, hexamethyldisilazane, hexamethylcyclotrisilazane, dimethylaminotrimethylsilane, trimethylvinylsilane, tetramethoxysilane, aminopropyltriethoxysilane, octadecyldiethoxymethylsilane, hexamethyldisilane and divinyltetramethyldisiloxane.

[0029] Compounds of formulae (I) and (II) having no double bond or triple bond are preferably used because a layer is formed slowly so that the resulting layer is more homogeneous, compared with compounds having double bonds or triple bonds.

[0030] The thickness of plasma polymerization layer 3 is preferably 100 to 3000 angstroms, most preferably 500 to 1000 angstroms.

[0031] Plasma polymerization layer 3 can be formed by plasma treatment to a resulting plasma layer with a polymeric or non-polymeric monomer. Examples of such non-polymeric monomer material include nitrogen, ammonium, hydrazine, hydrogensulfide, hydrogendisulfide, oxygen, hydrogen, water, halogen gas, and rare gas (e.g., argon, neon, helium, krypton, and xenon).

[0032] Furthermore, a mixture of various kinds of monomer materials can be used as a monomer material. Plasma polymerization layer 3 can also be formed by lamination techniques and optionally using a mixture as a monomer material.

[0033] Plasma polymerization layer 3 of the present invention has the following advantages:

1) The layer is pinhole-free, amorphous, and dense.
2) A thin homogeneous layer down to about 500 angstroms can be made, which exhibits extremely little fluctuation

in its refractive index.

3) By changing the kind of plasma gas, not only a change in the thickness of the layer but also surface modification and surface improvement, such as introduction of functional groups, and control of the density of the functional groups to be introduced can be attained.

4) The layer can be formed in combination with semiconductor techniques since it is formed under dry conditions.

5) The layer has excellent drug tolerance, heat tolerance, mechanical properties, and stability.

[0034] Furthermore, in the case of a sensor chip for SPR, in which a metal thin-film is essential, the metal thin-film and the plasma polymerization layer can be formed in the same chamber. Thus, the manufacturing process can be simplified.

[0035] It is also advantageous to attain surface improvement, such as introduction of a functional group, by further exposing a resulting plasma polymerization layer to plasma treatment with a non-polymeric or polymeric monomer. The plasma polymerization treatment is intended to include a treatment with not only a non-polymeric monomer and an inactive monomer but also a polymeric monomer.

[0036] Physiologically active substance 4 to be immobilized is not particularly limited, provided it reacts interacts with a target substance to be measured. Examples of physiologically active substance 4 include nucleic acids (e.g., DNA, RNA, and PNA); non-immune proteins (e.g., avidin (streptoavidin), biotin or a receptor); immunoglobulin-binding proteins (e. g., protein A, protein G and a rheumatoid factor (RF)); sugar-binding proteins (e.g., lectin); sugar-recognizing sugar chains; fatty acids or fatty acid esters (e.g., stearic acid, alachidic acid, behenic acid, ethyl stearate, ethyl arachidate, and ethyl behanate); polypeptides or oligopeptides having ligand binding activity; immune proteins (e.g., an antibody); and enzyme.

[0037] When an antibody is used as physiologically active substance 4, the antibody can be immobilized on the surface of plasma polymerization layer 3 through its Fc fragment and a monomolecular layer of the antibody is formed. However, since the sensitivity and the reaction rate decrease as Fab fragments of the antibody are separated from plasma polymerization layer 3, Fab fragments (Fig. 3 (a)) or $F(ab')_2$ fragments (Fig. 3 (b)) can be immobilized directly on plasma polymerization layer 3 as shown in Figure 3 to improve the sensitivity and the reaction rate.

[0038] The thickness of physiologically active substance 4 depends on the size of the physiologically active substance itself, but is preferably 100 to 3000 angstroms, most preferably 100 to 1000 angstroms.

[0039] In the present invention, the physiologically active substance can be immobilized on the plasma polymerization layer through linking agents.

[0040] Figure 4 is a schematic illustration showing one embodiment of the measuring chip according to the present invention. The measuring chip has covalent bond layer 6 between plasma polymerization layer 3 and physiologically active substance 4. Substance 4 is immobilized on plasma polymerization layer 3 via covalent layer 6. The covalent bond can be formed with a cross-linking reagent or a condensation reagent.

[0041] The cross-linking reagent or a condensation reagent is not particularly restricted, provided it can covalently and firmly immobilize substance 4. They can be used alone or in combination.

[0042] Examples of such cross-linking reagents include glutaraldehyde, periodic acid, N-succinimidyl-2-maleimidoacetic acid, N-succinimidyl-4-maleimidobutyric acid, N-succinimidyl-6-maleimidohexanic acid, N-succinimidyl-4-maleimidomethylcyclohexan-1-carboxylic acid, N-sulfosuccinimidyl-4-maleimidomethylcyclohexane-1-carboxylic acid, N-succinimidyl-4-maleimidomethylbanzoic acid, N-succinimidyl-3-maleimidobenzoic acid, N-sulfosuccinimidyl-3-maleimidobenzoic acid, N-succinimidyl-4-maleimidophenyl-4-butyric acid, N-sulfosuccinimidyl-4-maleimidophenyl-4-butyric acid, N,N'-oxydimethylene-dimaleimide, N,N'-o-phenylene-dimaleimide, N,N'-m-phenylene-dimaleimide, N,N'-p-phenylene-dimaleimide, N,N'-hexamethylene-dimaleimide, N-succinimidylmaleimidocarboxylic acid, N-succinimidyl-S-acetylmercaptoacetic acid, N-succinimidyl-3-(2-pyridyldithio)propionate, S-acetylmercaptosuccinic anhydride, methyl-3-(4'-dithiopyridyl)propionimidate, methyl-4-mercaptobutylimidate, methyl-3-mercaptopropionimidate, iminothiolene, *o*-carboxymethyl-hydroxylamine, azodiphenylpilmaleido, bis(sulfosuccinimidyl)sperate, 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene, 4,4'-difluoro-3,3'-dinitrodiphenylsulfon, 1,5-difluoro-2,4-dinitrobenzene, p-phenylenediisothiocyanate, dimethyladipimidate, dimethylpimelimidate, dimethylsuberimidate, p-azidophenacylbromide, p-azidophenylglyoxal, N-hydroxysuccinimidyl-4-azidobenzoate, 4-fluoro-3-nitrophenylazide, methyl-4-azidobenzoimidate, N-5-azido-2-nitrobenzoyloxysuccinimide, N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, 1,4-benzoquinone, N-succinimidyl-3-(2'-pyridyldithio)propionate, N-(4-maleimidobutyloxy)sulfosuccinimide sodium salt, N-(6-maleimidocaproyloxy)sulfosuccinimide sodium salt, N-(8-maleimidocaproyloxy)sulfosuccinimide sodium salt, N-(11-maleimidoundecanoyloxy)sulfosuccinimide sodium salt, N-[2-(1-piperazinyl)ethyl]maleimide bichloric acid, bisdiazobenzidine, hexamethylenediisocyanate, toluenediisocyanate, hexamethylenediisothiocyanate, N,N'-ethylenebismaleinimide, N,N'-polymethylenebisiodoacetamide, 2,4-dinitrobenzenesulfonate sodium salt, and diazo compounds. Glutaraldehyde is preferable as a cross-linking reagent.

[0043] Examples of such condensation reagents include carbodiimide derivatives represented by formula RN=C=NR (or R'), N-hydroxysuccinimide, tri-n-butylamine, butyl chloroformate, and isobutyl isocyanide.

[0044] By introducing covalent layer 6 to the measuring cell to firmly mobilize physiologically active substance 4 via covalent bonds, substance 4 can be maintained immobilized when the measuring cell is washed, which enables the cell to be used for repetitive measurements for another advantageous feature. The thickness of covalent layer 6 is preferably 10 to 100 angstroms, most preferably 10 to 20 angstroms.

[0045] The physiologically active substance can also be immobilized by hydrophobic bond, by integrating substance 4 into a plasma polymerization layer or by an additional plasma treatment.

[0046] A preferred group of the measuring chip according to the present invention is a measuring chip comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and an immune protein or enzyme immobilized on the surface of said plasma polymerization layer, wherein said plasma polymerization layer comprises a monomer material selected from the group consisting of pyridine, triethylamine, diethylamine, allylamine, acrylamide, aniline, acrylonitrile, 1,2,4-triazole, 5-amino-1H-tetrazole, and acetonitrile,

[0047] Another preferred group of the measuring chip according to the present invention is a measuring chip comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and an immune protein or enzyme immobilized on the surface of said plasma polymerization layer, wherein said plasma polymerization layer comprises a monomer material selected from the group consisting of pyridine, triethylamine, diethylamine, allylamine, acrylamide, aniline, acrylonitrile, 1,2,4-triazole, 5-amino-1H-tetrazole, and acetonitrile and wherein said immune protein or enzyme is immobilized on said plasma polymerization layer through a cross-linking reagent or a water-soluble condensation reagent.

[0048] A cross-linking reagent for the preferred group above can be selected from the group consisting of glutaraldehyde, N-succinimidyl-4-maleimidomethylbanzoic acid, N-succinimidyl-3-maleimidobenzoic acid, N-succinimidyl-4-maleimidophenyl-4-butyric acid, N,N'-oxydimethylene-dimaleimide, N,N'-m-phenylene-dimaleimide, N,N'-p-phenylene-dimaleimide, N,N'-hexamethylene-dimaleimide, N-succinimidylmaleimidocarboxylic acid, N-succinimidyl-S-acetylmercaptoacetic acid, N-succinimidyl-3-(2-pyridyldithio)propionate, S-acetylmercaptosuccinic anhydride, methyl-3-(4'-dithiopyridyl)propionimidate, methyl-4-mercaptobutylimidate, methyl-3-mercaptopropionimidate, iminothiolene, o-carboxymethyl-hydroxylamine, azodiphenylpilmaleido, bis(sulfosuccinimidyl)sperate, 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene, 4,4'-difluoro-3,3'-dinitrodiphenylsulfon, 1,5-difluoro-2,4-dinitrobenzene, p-phenylenediisothiocyanate, dimethyladipimidate, dimethylpimelimidate, dimethylsuberimidate, p-azidophenacylbromide, p-azidophenylglyoxal, N-hydroxysuccinimidyl-4-azidobenzoate, 4-fluoro-3-nitrophenylazide, methyl-4-azidobenzoimidate, N-5-azido-2-nitrobenzoyloxysuccinimide, N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, 1,4-benzoquinone, N-succinimidyl-3-(2'-pyridyldithio)propionate, bisdiazobenzidine, hexamethylenediisocyanate, toluenediisocyanate, hexamethylenediisothiocyanate, N,N'-ethylenebismaleinimido, N,N'-polymethylenebisiodoacetoamide, and diazo compounds; or said condensation reagent is one or more compounds selected from the group consisting of carbodiimide derivatives represented by RN=C=NR (or R'), N-hydroxysuccinimide, tri-n-butylamine, butyl chloroformate, and isobutyl isocyanide.

[0049] The measuring chip according to the present invention can be formed as follows:

[0050] First, metal thin-film 2 is formed on transparent substrate 1. Metal thin-film 2 can be formed by conventional methods such as sputtering, CVD, PVD, or vacuum evaporation.

[0051] Second, plasma polymerization layer 3 is formed on metal thin-film 2. Plasma polymerization layer 3 can be formed by plasma polymerization using a plasma polymerization apparatus. The rate of plasma formation is preferably 100 to 3000 angstroms/min, most preferably 500 to 1000 angstroms/min. If the rate exceeds 3000 angstroms/min, it becomes difficult to obtain a smooth plasma polymerization layer. More specifically, the plasma polymerization can be preferably carried out at a monomer material flow rate of 0.05 to 100 sccm at a room temperature or at a temperature of 10 to 20°C at a pressure between 1.0×10^{-2} and 1.0×10^{2} Pa using a discharge power of 20 to 300 W at a discharge frequency of 10 MHz or 13.56 MHz. However, polymerization conditions are not restricted to the conditions above.

[0052] After formation of plasma polymerization layer 3, physiologically active substance 4 is finally immobilized on plasma polymerization layer 3. Immobilization can be done by conventional methods. For example, a specified amount of physiologically active substance 4 can be immobilized by contacting it with plasma polymerization layer 3 for a specified period of time. If the measuring cell is a flow-cell type, a specified volume of the physiologically active substance 4 can be immobilized by contacting it with plasma polymerization layer 3 by pouring a specified volume for a specified period of time.

[0053] When an antibody is used as a physiologically active substance and its Fab fragment is immobilized directly on plasma polymerization layer 3, the same treatment can be done after the antibody is partly digested with papain. On the other hand, when the $F(ab')_2$ fragment is immobilized directly on plasma polymerization layer 3, the same treatment can be done after the antibody is partly digested with pepsin.

[0054] When covalent bond layer 6 is formed, a cross-linking reagent or a condensation reagent is allowed to be in contact with plasma polymerization layer 3 in the same manner as with active substance 4, after which substance 4 can be immobilized.

[0055] The measuring cell for a surface plasmon resonance sensor according to the present invention comprises the measuring chip. The measuring chip can be mounted on an optical part to be optically analyzed. The term "optical part"

as used herein refers to a part where a light is projected and an evanescent wave and a surface plasmon can be induced.

[0056] The surface plasmon resonance biosensor according to the present invention comprises the measuring cell.

[0057] Figure 5 is a schematic view of one embodiment of the surface plasmon resonance biosensor according to the present invention. The surface plasmon resonance biosensor has cartridge block 7, light source 8, and detector 9 and measuring chip 10 is mounted on cartridge block 7. The upper side of cartridge block 7 has a hollow and this hollow and measuring chip 10 construct measuring cell 71.

[0058] The body of measuring chip 10 comprises a transparent substrate, and a layer comprising a metal thin-film, a plasma polymerization layer formed under said metal film. A physiologically active substance is immobilized on the surface of said plasma polymerization layer facing the hollow of cartridge block 7. Measuring cell 71 is constructed from the hollow of cartridge block 7 and measuring chip 10; and cartridge block 7 has flow routes 72 and 73 providing passages to the outside of measuring cell 71 and cartridge block 7, which makes measuring cell 71 a flow-cell type. However, the present invention is not restricted to this type and a batch type cell can also be used. Using measuring cell 71 of this flow-cell type, a sample can be measured either continuously or intermittently. In this sensor, the sample flows into measuring cell 71 via flow route 72 and is discharged after measurement via flow route 73. The flow rate of the sample is preferably 0.5 to 5 μl/min. The flow rate is controlled, for example, using a computer-operated pump.

[0059] Monochromatic light (incident light 80) is irradiated from light source 8 toward the optical part of measuring chip 10 and its reflected light 90, which is reflected by metal thin-film 2 set on the reverse side of measuring chip 10, reaches detector 9. Detector 9 can detect the intensity of reflected light 90. Light source 8 and detector 9 are not particularly restricted, and can be any types customarily used for a surface plasmon resonance biosensor. In the sensor according to the present invention, the incident light is wedge-shaped and the light reflected in different directions can be measured simultaneously. However, the present invention is not restricted to this type of sensor. The configuration of this type does not require a mobile part, thereby producing excellent stability and durability, and enabling real time measurement of samples as well.

[0060] The configuration as described above yields a reflected light intensity curve that forms a trough relative to a given angle of incidence (see Figure 6). The trough in the reflected light intensity curve is due to surface plasmon resonance. Namely, when light is totally reflected at the interface between the transparent substrate and the exterior of measuring chip 10, a surface wave known as an evanescent wave is generated at the interface and a surface wave known as a surface plasmon is also generated on the metal thin-film. Resonance occurs when the wave number of these two surface waves coincides and a part of light energy is consumed to excite the surface plasmon, resulting in a decrease in the intensity of the reflected light. The wave number of the surface plasmon is affected by the refractive index of the medium proximate to the surface of the metal thin-film. Therefore, when the refractive index of the medium changes due to an interaction between the substance to be measured and the physiologically active substance, a surface plasmon resonance is induced to change the angle of incidence. Thus, a change in the concentration of the substance to be measured can be perceived by a shift of the trough in the reflected light intensity curve. The change in the angle of incidence is called a resonance signal and a change of 10^{-4} degree is expressed as 1 RU. In the surface plasmon resonance biosensor of this example, highly effective and reliable measurement can be done if measuring chip 10 is made to be freely attachable and detachable and disposable. Furthermore, if a covalent bond layer is provided between the plasma polymerization layer and the physiologically active substance, measuring chip 10 can be used repeatedly by washing the inside of measuring cell 71, resulting in a decrease in the cost.

[0061] The surface plasmon resonance biosensor of the present invention can be used for quantitative or qualitative analysis, identification of a target substance present in a sample.

EXAMPLE

[0062] The present invention is further illustrated by the following Examples that are not intended as a limitation of the invention.

Example 1

[0063] A measuring chip having layers shown in Figure 1 on an optical recognition part was constructed.

[0064] A glass plate with a thickness of 0.15 mm (18 mm x 18 mm) was used for a transparent substrate. A chrome layer and then a gold layer were deposited on this transparent substrate by sputtering. The sputtering was carried out at 100 W for 40 seconds for the chrome layer and at 100 W for 2 minutes and 30 seconds for the gold layer. The resulting chrome layer was 40 angstroms thick and the resulting gold layer was 500 angstroms thick.

[0065] A plasma polymerization layer was formed on the metal layers. An apparatus as shown in Figure 7 was used for plasma polymerization. Ethanedithiol was used as a monomer material for the plasma polymerization layer to introduce a thiol group. Conditions for plasma polymerization were as follows:

Flow volume of monomer material: 15 sccm
Temperature: 15 °C
Pressure: 4.7 Pa
Discharge electric power: 20 W
Discharge frequency: 10 MHz, FM modulation
Duration of discharge: 60 seconds.

[0066] Under the conditions described above, a thiol group was introduced on the surface of plasma polymerization layer. The sensor chip with the introduced thiol group was mounted on the cartridge block of the surface plasmon resonance biosensor and maleimidized avidin (see "Ultrahigh Sensitivity Enzyme Immunoassay" by Eiji Ishikawa) was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for immobilization on the thiol group on the plasma polymerization layer for 60 minutes. 50 µl of 10 µM-biotinized DNA were then poured and the probe DNA was immobilized via the avidin for 10 minutes. A DNA (7.5×10^{-7} M) having a DNA sequence complementary to this probe DNA was introduced and after the reaction, a signal of about 500 RU was obtained.

Concentration of Complementary DNA (µM)	0.00075	0.0075	0.075	0.75	7.5	75
RU	10	25	100	500	1000	1100

[0067] It was confirmed by an XPS analysis that the resulting membrane has a mercapto group.

[0068] Figure 6 shows the reflected light intensity curve before and after the formation of the plasma polymerization layer, which show the intensity of reflected light corresponding to the angle of incidence θ). Figure 6 shows that the plasma polymerization layer is formed on the surface of the gold layer. The thickness of the plasma polymerization layer can be estimated from Δθ.

Example 2

[0069] The same apparatus and method as in Example 1 were used.

[0070] Acetonitrile was used as a monomer material for the plasma polymerization layer. Conditions for plasma polymerization were as follows:

Flow volume of monomer material: 1.5 sccm + Ar dilution 15 (sccm)
Temperature: room temperature
Pressure: 4.7 Pa
Discharge electric power: 80 W
Discharge frequency: 13.56 MHz
Duration of discharge: 15 seconds.

[0071] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and avidin (concentration: 20 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. 10 µM biotin-labeled probe RNA were then poured at a flow rate of 1 µl/min to immobilize the probe RNA for 10 minutes. DNA (7.5×10^{-7} M) having a DNA sequence complementary to this probe RNA was introduced and after the reaction, a signal of about 500 RU was obtained.

Concentration of complementary DNA (µM)	0.00075	0.0075	0.075	0.75	7.5	75
RU	8	20	80	400	800	880

[0072] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 3

[0073] The same apparatus and method as in Example 1 were used.

[0074] Conditions for plasma polymerization layer formation were the same as in Example 2.

[0075] Under the conditions described above, a plasma polymerization layer was formed.

[0076] The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and streptoavidin (concentration: 20 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. 10 μM biotin-labeled probe RNA was then poured at a flow rate of 1 μl/min for 10 minutes to immobilize the probe RNA. DNA (7.5 x 10^{-7} M) having a DNA sequence complementary to this probe RNA was introduced and after the reaction, a signal of about 375 RU was obtained.

Concentration of complementary DNA (μM)	0.00075	0.0075	0.075	0.75	7.5	75
RU	7.5	18.75	75	375	750	825

[0077] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 4

[0078] The same apparatus and method as in Example 1 were used.

[0079] Conditions for plasma polymerization layer formation were the same as in Example 2 except that propargylamine was used as a monomer material.

[0080] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 0.4 M N-ethyl-N'-(3-dimethylaminopropyl)carbodiimide was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and avidin (concentration: 20 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. 10 μM biotin-labeled probe RNA was then poured at a flow rate of 1 μl/min for 10 minutes to immobilize the probe RNA. DNA (7.5 x 10^{-7} M) having a DNA sequence complementary to this probe RNA was introduced and after the reaction, a signal of about 450 RU was obtained.

Concentration of complementary DNA (μM)	0.00075	0.0075	0.075	0.75	7.5	75
RU	0.9	22.5	90	450	900	990

[0081] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 5

[0082] The same apparatus and method as in Example 1 were used.

[0083] Conditions for plasma polymerization layer formation were the same as in Example 4.

[0084] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and protein A (concentration: 400 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. An anti-HSA antibody (concentration: 400 μl/ml) was then poured at a flow rate of 1 μl/min for 10 minutes to immobilize the antibody. An HSA antigen (10 μg/ml) complementary to this anti-HSA antibody was introduced and after the reaction, a signal of about 250 RU was obtained.

Concentration of HSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	5	12.5	50	250	500	550

[0085] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 6

[0086] The same apparatus and method as in Example 1 were used.

[0087] Conditions for plasma polymerization layer formation were the same as in Example 4.

[0088] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and protein G (concentration: 400 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. An anti-BSA antibody (concentration: 400 μl/ml) was then poured at a flow rate of 1 μl/min for 10 minutes to immobilize the antibody. A BSA antigen (10 μg/ml) complementary to this anti-BSA antibody was introduced and after the reaction, a signal of about 225 RU was obtained.

Concentration of BSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	4.5	11.25	45	225	450	495

[0089] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 7

[0090] The same apparatus and method as in Example 1 were used.

[0091] Conditions for plasma polymerization layer formation were the same as in Example 4.

[0092] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and mannose-binding lectin (concentration: 200 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes.

[0093] A sugar (10 μg/ml) complementary to this mannose-binding lectin was introduced and after the reaction, a signal of about 200 RU was obtained.

Concentration of sugar (μg/ml)	0.01	0.1	1	10	100	1000
RU	4	10	40	200	400	440

[0094] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 8

[0095] The same apparatus and method as in Example 1 were used.

[0096] Conditions for plasma polymerization layer formation were the same as in Example 4 except that pyridine was used as a monomer material.

[0097] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and an anti-BSA antibody (concentration: 400

μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. A BSA antigen (10 μg/ml) complementary to this anti-BSA antibody was introduced and after the reaction, a signal of about 187.5 RU was obtained.

Concentration of BSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	3.75	9.375	37.5	187.5	375	412.5

[0098] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 9

[0099] The same apparatus and method as in Example 1 were used.

[0100] Conditions for plasma polymerization layer formation were the same as in Example 8 except that acrylonitrile was used as a monomer material.

[0101] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 μl/min for 10 minutes and an anti-BSA antibody (concentration: 400 μg/ml) was also poured at a flow rate of 5 μl/min to immobilize for 60 minutes. A BSA antigen (10 μg/ml) complementary to this anti-BSA antibody was introduced and after the reaction, a signal of about 200 RU was obtained.

Concentration of BSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	4	10	40	200	400	440

[0102] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 10

[0103] The same apparatus and method as in Example 1 were used.

[0104] Conditions for plasma polymerization layer formation were the same as in Example 9 except that ethanethiol was used as a monomer material.

[0105] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor and maleimidized anti-BSA antibody was poured through a flow route at a flow rate of 5 μl/min to immobilize for 60 minutes. A BSA antigen (10 μg/ml) complementary to this anti-BSA antibody was introduced and after the reaction, a signal of about 200 RU was obtained.

Concentration of BSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	4	10	40	200	400	440

[0106] It was confirmed by the XPS analysis that the resulting membrane has a mercapto group.

Example 11

[0107] The same apparatus and method as in Example 1 were used.

[0108] Conditions for plasma polymerization layer formation were the same as in Example 10 except that thiophene was used as a monomer material.

[0109] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor and maleimidized anti-BSA antibody was

poured through a flow route at a flow rate of 5 µl/min to immobilize for 60 minutes. A BSA antigen (10 µg/ml) complementary to this anti-BSA antibody was introduced and after the reaction, a signal of about 187.5 RU was obtained.

Concentration of BSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	3.75	9.375	37.5	187.5	375	412.5

[0110] It was confirmed by the XPS analysis that the resulting membrane has a mercapto group.

Example 12

[0111] The same apparatus and method as in Example 1 were used.
[0112] Conditions for plasma polymerization layer formation were the same as in Example 11 except that acetonitrile was used as a monomer material.
[0113] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. HSA antigen (10 µg/ml) complementary to this anti-HSA antibody was introduced and after the reaction, a signal of about 250 RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	5	10	50	250	500	550

[0114] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 13

[0115] The same apparatus and method as in Example 1 were used.
[0116] Conditions for plasma polymerization layer formation were the same as in Example 12.
[0117] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and the Fab fragment of an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes.
[0118] A HSA antigen (10 µg/ml) complementary to this Fab fragment of the anti-HSA antibody was introduced and after the reaction, a signal of about 275 RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	5.5	11	55	275	550	605

[0119] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 14

[0120] The same apparatus and method as in Example 1 were used.
[0121] Conditions for plasma polymerization layer formation were the same as in Example 13.
[0122] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a

flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and the $F(ab)_2$ fragment of an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes.

[0123] A HSA antigen (10 µg/ml) complementary to this $F(ab)_2$ fragment of the anti-HSA antibody was introduced and after the reaction, a signal of about 300 RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	6	12	60	300	600	660

[0124] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 15

[0125] The same apparatus and method as in Example 1 were used. Conditions for plasma polymerization layer formation were as follows:

(1) Monomer: hexadiene

Flow volume of monomer material: 1.5 sccm + Ar dilution 15 (sccm)
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 80 W
Discharge frequency: 13.56 MHz
Duration of discharge: 15 seconds;

(2) Monomer: ethylenediamine

Flow volume of monomer material: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 80 W
Discharge frequency: 13.56 MHz
Duration of discharge: 5 seconds.

The targeted surface was obtained by the two-step process above.

[0126] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. A HSA antigen (10 µg/ml) complementary to this anti-HSA antibody was introduced and after the reaction, a signal of about 250 RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	5	10	50	250	500	550

[0127] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 16

[0128] The same apparatus and method as in Example 1 were used. Conditions for plasma polymerization layer formation were as follows:

(1) Monomer: hexamethyldisiloxane

Flow volume of monomer material: 1.5 sccm + Ar dilution 15 (sccm)
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 80 W
Discharge frequency: 13.56 MHz
Duration of discharge: 15 seconds;

(2) Monomer: ethylenediamine

Flow volume of monomer material: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 80 W
Discharge frequency: 13.56 MHz
Duration of discharge: 5 seconds.

The targeted surface was obtained by the two-step process above.

[0129] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. A HSA antigen (10 µg/ml) complementary to this anti-HSA antibody was introduced and after the reaction, a signal of about 225RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	4.5	9	45	225	450	495

[0130] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 17

[0131] The same apparatus and method as in Example 1 were used.

[0132] Conditions for plasma polymerization layer formation were the same as in Example 2 except that propylamine was used as a monomer material.

[0133] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 5% glutaraldehyde was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and avidin (concentration: 20 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. 10 µM biotin-labeled probe RNA was poured at a flow rate of 1 µl/min to immobilize the probe RNA for 10 minutes. DNA (7.5×10^{-7} M) having a DNA sequence complementary to this probe RNA was introduced and after the reaction, a signal of about 400 RU was obtained.

Concentration of Complementary DNA (µM)	0.00075	0.0075	0.075	0.75	7.5	75
RU	8	20	80	400	800	880

[0134] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 18

[0135] The same apparatus and method as in Example 1 were used.

[0136] Conditions for plasma polymerization layer formation were as follows:

(1) Monomer: propargyl alcohol

Flow volume of monomer material: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 20 W
Discharge frequency: 13.56 MHz
Duration of discharge: 15 seconds;

(2) Monomer: oxygen (plasma treatment)

Flow volume of monomer material: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 20 W
Discharge frequency: 13.56 MHz
Duration of discharge: 5 seconds.

The targeted surface was obtained by the two-step process above.

[0137] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, a 0.5 M carbodiimide solution was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and an anti-HSA antibody (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. A HSA antigen (10 µg/ml) complementary to this anti-HSA antibody was introduced and after the reaction, a signal of about 250 RU was obtained.

Concentration of HSA antigen (µg/ml)	0.01	0.1	1	10	100	1000
RU	5	10	50	250	500	550

[0138] It was confirmed by the XPS analysis that the resulting membrane has a carboxyl group.

Example 19

[0139] The same apparatus and method as in Example 1 were used.

[0140] Conditions for plasma polymerization layer formation were the same as in Example 2 except that propagylamine was used as a monomer material.

[0141] Under the conditions described above, a plasma polymerization layer was formed. The sensor chip was mounted on the cartridge block of the surface plasmon resonance biosensor, 0.5 M carbodiimide was poured through a flow route into the measuring cell at a flow rate of 5 µl/min for 10 minutes and behenic acid (concentration: 400 µg/ml) was also poured at a flow rate of 5 µl/min to immobilize for 60 minutes. Skatole (10 µg/ml) complementary to this behenic acid was introduced and after the reaction, a signal of about 225 RU was obtained.

Concentration of skatole (µg/ml)	0.01	0.1	1	10	100	1000
RU	4.5	9	45	225	450	495

[0142] It was confirmed by the XPS analysis that the resulting membrane has a primary amine.

Example 20

[0143] Example 20 shows a formation of hydrophobic bond.

[0144] A layer comprising chrome and gold was formed on a transparent substrate (glass plate) by sputtering. A plasma polymerization layer in which trifluoroethylene was used as a monomer was then formed on the resulting metal layer under the following conditions:

Flow volume: 1.5 sccm
Temperature: room temperature
Pressure: 5 Pa
Discharge electric power: 50 W
Discharge frequency: 13.56 MHz
Duration of discharge: 30 seconds.

[0145] The plasma polymerization layer obtained under the conditions described above was hydrophobic. An anti-HSA antibody (concentration: 100 μg/ml) was allowed to flow at a flow rate of 5 μl/min for 60 minutes to immobilize the antibody via hydrophobic bond. HSA at a specified concentration was further reacted with this antibody-immobilized plasma polymerization layer. The following results were obtained.

Concentration of HSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	3	6	30	150	300	330

Example 21

[0146] Example 21 shows an inclusion of an antibody by plasma polymerization.

[0147] A layer comprising chrome and gold was formed on a transparent substrate (glass plate) by sputtering. A plasma polymerization layer in which propargyl alcohol was used as a monomer was then formed on the resulting metal layer under the following conditions:

Flow volume: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 20 W
Discharge frequency: 13.56 MHz
Duration of discharge: 15 seconds.

[0148] The plasma polymerization layer obtained under the conditions described above was highly hydrophilic. An antibody solution (concentration: 100 μg/ml) was spread evenly on this propargyl alcohol plasma polymerization layer and after drying, plasma treatment was further carried out on this surface under the following conditions:

Flow volume: 1.5 sccm
Temperature: room temperature
Pressure: 1.6 Pa
Discharge electric power: 20 W
Discharge frequency: 13.56 MHz
Duration of discharge: 8 seconds.

[0149] HSA at a specified concentration was reacted with the membrane in which the antibody was thus integrated and immobilized by plasma treatment. The following results were obtained, from which a calibration curve could be drawn.

Concentration of HSA antigen (μg/ml)	0.01	0.1	1	10	100	1000
RU	5	10	50	250	500	550

*Flow rate for HSA: 5 μl/min.

[0150] The following pages 32 to 42 of the description represent preferred embodyments 1 to 63 of the invention, wherein the term "claims" is to be read as "embodiments".

1. A measuring chip for a surface plasmon resonance sensor comprising a metal layer and one or more plasma polymerization layers formed on said metal layer.

2. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a nucleic acid immobilized on the surface of said plasma polymerization layer.

3. A measuring chip for a surface plasmon resonance sensor according to claim 2, wherein said nucleic acid is DNA, RNA or PNA.

4. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a non-immune protein immobilized on the surface of said plasma polymerization layer.

5. A measuring chip for a surface plasmon resonance sensor according to claim 4, wherein said non-immune protein is either avidin, streptoavidin, biotin or a receptor.

6. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and an immunoglobulin-binding protein immobilized on the surface of said plasma polymerization layer.

7. A measuring chip for a surface plasmon resonance sensor according to claim 6, wherein said immunoglobulin-binding protein is protein A, protein G, or a rheumatoid factor.

8. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a sugar-binding protein immobilized on the surface of said plasma polymerization layer.

9. A measuring chip for a surface plasmon resonance sensor according to claim 8, wherein said sugar-binding protein is lectin.

10. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a sugar-recognizing sugar chain immobilized on the surface of said plasma polymerization layer.

11. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a fatty acid or a fatty acid ester immobilized on the surface of said plasma polymerization layer.

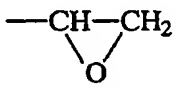
12. A measuring chip for a surface plasmon resonance sensor according to claim 11, wherein said fatty acid or fatty acid ester is either stearic acid, alachidic acid, behenic acid, ethyl stearate, ethyl arachidate, or ethyl behanate.

13. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a polypeptide or oligopeptide having ligand-binding activity immobilized on the surface of said plasma polymerization layer.

14. A measuring chip for a surface plasmon resonance sensor according to claim 13, wherein said polypeptide or oligopeptide is produced by using genetic engineering techniques or chemical synthesis methods.

15. A measuring chip according to any one of claims 1 to 14, which further comprises an optically transparent substrate on which said metal layer is formed.

16. A measuring chip according to any one of claims 1 to 15, wherein said plasma polymerization layer comprises a compound having one or more groups selected from the group consisting of -COOH, -CHO, -SH, $-NH_2$, -OH, =NH, $-CONH_2$, -NCO, $-CH=CH_2$, =C=O, and



17. A measuring chip according to any one of claims 1 to 15, wherein said plasma polymerization layer consists of two or more layers.

18. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer is a compound containing nitrogen.

19. A measuring chip according to claim 18, wherein said compound containing nitrogen is CH_3-$(CH_2)_n$-NH_2 (wherein n is an integer from 1 to 6) and/or NH_2-$(CH_2)_n$-NH_2 (wherein n is an integer from 1 to 6).

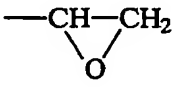
20. A measuring chip according to claim 18, wherein said compound containing nitrogen is selected from the group consisting of pyridine, ethylenediamine, hexamethylenediamine, n-propylamine, monoethylamine, triethylamine, diethylamine, allylamine, acrylamide, aniline, acrylonitrile, 1,2,4-triazole, 5-amino-1H-tetrazole, propargylamine and acetonitrile.

21. A measuring chip according to any one of claims 1 to 15, wherein a monomer material of said plasma polymerization layer is a compound containing sulfur.

22. A measuring chip according to claim 21, wherein said compound containing sulfur is selected from the group consisting of dimethyl sulfide, methyl disulfide, ethanethiol, ethanedithiol, thiophen, mercaptoethanol and dithreitol.

23. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer is a compound containing a halogen.

24. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer comprises a compound having one or more groups selected from the group consisting of -COOH, -CHO, -SH, $-NH_2$, -OH, =NH, $-CONH_2$, -NCO, - $CH=CH_2$, =C=O and



25. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer is a compound having $-C{\equiv}CCH_2OH$.

26. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer is a carbohydrate compound comprising C and H.

27. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of a plasma polymerization layer is an organic metal compound.

28. A measuring chip according to claim 27, wherein said organic metal compound is an organic silicon compound.

29. A measuring chip according to claim 28, wherein said organic silicon compound is selected from the group consisting of tetramethylsilane, tetramethyldisiloxane, hexamethyldisiloxane, hexamethyldisilazane, hexamethylcyclotrisilazane, dimethylaminotrimethylsilane, trimethylvinylsilane, tetramethoxysilane, aminopropyltriethoxysilane, octadecyldiethoxymethylsilane, hexamethyldisilane, and divinyltetramethyldisiloxane.

30. A measuring chip according to any one of claims 1 to 15, wherein plasma treatment is applied to said plasma polymerization layer with a polymeric or non-polymeric monomer.

31. A measuring chip according to claim 30 wherein said non-polymeric monomer material is selected from the group consisting of nitrogen, ammonium, hydrazine, hydrogensulfide, hydrogendisulfide, oxygen, hydrogen, water, halogen gas, and rare gas.

32. A measuring chip according to any one of claims 1 to 15, wherein said monomer material of plasma polymerization layer is a mixture of two or more of the substances claimed in claims 18 to 31.

33. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and an immune protein or enzyme immobilized on the surface of said plasma polymerization layer, wherein said plasma polymerization layer comprises a monomer material selected from the group consisting of pyridine, triethylamine, diethylamine, allylamine, acrylamide, aniline, acrylonitrile, 1,2,4-triazole, 5-amino-1H-tetrazole, and acetonitrile.

34. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and an immune protein or enzyme immobilized on the surface of said plasma polymerization layer, wherein said plasma polymerization layer comprises a monomer material selected from the group consisting of the compounds claimed in any one of claims 21 to 32.

35. A measuring chip according to claim 33 or 34, wherein said immune protein is an antibody.

36. A measuring chip according to claim 33 or 34, wherein said immune protein is a Fab fragment of an antibody.

37. A measuring chip according to claim 33 or 34, wherein said immune protein is a F(ab)2 fragment of an antibody.

38. A measuring chip according to claim 2 or 3, wherein said nucleic acid is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

39. A measuring chip according to claim 4 or 5, wherein said non-immune protein is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

40. A measuring chip according to claim 6 or 7, wherein said immunoglobulin-binding protein is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

41. A measuring chip according to claim 8 or 9, wherein said sugar-binding protein is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

42. A measuring chip according to claim 10, wherein said sugar-recognizing sugar chain is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

43. A measuring chip according to claim 11 or 12, wherein said fatty acid or fatty acid ester is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

44. A measuring chip according to claim 13, wherein said polypeptide or oligopeptide is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

45. A measuring chip according to any one of claims 38 to 44, wherein said cross-linking reagent is one or more compounds selected from the group consisting of glutaraldehyde, periodic acid, N-succinimidyl-2-maleimidoacetic acid, N-succinimidyl-4-maleimidobutyric acid, N-succinimidyl-6-maleimidohexanic acid, N-succinimidyl-4-maleimidomethylcyclohexan-1-carboxylic acid, N-sulfosuccinimidyl-4-maleimidomethylcyclohexane-1-carboxylic acid, N-succinimidyl-4-maleimidomethylbanzoic acid, N-succinimidyl-3-maleimidobenzoic acid, N-sulfosuccinimidyl-3-maleimidobenzoic acid, N-succinimidyl-4-maleimidophenyl-4-butyric acid, N-sulfosuccinimidyl-4-maleimidophenyl-4-butyric acid, N,N'-oxydimethylene-dimaleimide, N,N'-o-phenylene-dimaleimide, N,N'-m-phenylene-dimaleimide, N,N'-p-phenylene-dimaleimide, N,N'-hexamethylene-dimaleimide, N-succinimidylmaleimidocarboxylic acid, N-succinimidyl-S-acetylmercaptoacetic acid, N-succinimidyl-3-(2-pyridyldithio)propionate, S-acetylmercaptosuccinic anhydride, methyl-3-(4'-dithiopyridyl)propionimidate, methyl-4-mercaptobutylimidate, methyl-3-mercaptopropionimidate, iminothiolene, *o*-carboxymethyl-hydroxylamine, azodiphenylpilmaleido, bis(sulfosuccinimidyl)sperate, 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene, 4,4'-difluoro-3,3'-dinitrodiphenylsulfon, 1,5-difluoro-2,4-dinitrobenzene, p-phenylenediisothiocyanate, dimethyladipimidate, dimethylpimelimidate, dimethylsuberimidate, p-azidophenacylbromide, p-azidophenylglyoxal, N-hydroxysuccinimidyl-4-azidobenzoate, 4-fluoro-3-nitrophenylazide, methyl-4-azidobenzoimidate, N-5-azido-2-nitrobenzoyloxysuccinimide, N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, 1,4-benzoquinone, N-succinimidyl-3-(2'-pyridyldithio)propionate, N-(4-maleimidobutyloxy)sulfosuccinimide sodium salt, N-(6-maleimidocaproyloxy)sulfosuccinimide sodium salt, N-(8-maleimidocaproyloxy)sulfosuccinimide sodium salt, N-(11-maleimidoundecanoyloxy)sulfosuccinimide sodium salt, N-[2-(1-piperazinyl)ethyl]maleimide bichloric acid, bisdiazobenzidine, hexamethylenediisocyanate, toluenediisocyanate, hexamethylenediisothiocyanate, N,N'-ethylenebismaleinimide, N,N'-polymethylenebisiodoacetamide, 2,4-dinitrobenzenesulfonate sodium salt, and diazo compounds; or said condensation reagent is one or more compounds selected from the group consisting of carbodiimide derivatives represented by RN=C=NR (or R'), N-hydroxysuccinimide, tri-n-butylamine, butyl chloroformate, and isobutyl isocyanide.

46. A measuring chip according to any one of claims 33 to 37, wherein said immune protein or enzyme is immobilized on said plasma polymerization layer through a cross-linking reagent or a water-soluble condensation reagent.

47. A measuring chip according to claim 46, wherein said cross-linking reagent is one or more compounds selected from the group consisting of glutaraldehyde, N-succinimidyl-4-maleimidomethylbanzoic acid, N-succinimidyl-3-maleimidobenzoic acid, N-succinimidyl-4-maleimidophenyl-4-butyric acid, N,N'-oxydimethylene-dimaleimide, N,N'-m-phenylene-dimaleimide, N,N'-p-phenylene-dimaleimide, N,N'-hexamethylene-dimaleimide, N-succinimidylmaleimidocarboxylic acid, N-succinimidyl-S-acetylmercaptoacetic acid, N-succinimidyl-3-(2-pyridyldithio)propionate, S-acetylmercaptosuccinic anhydride, methyl-3-(4'-dithiopyridyl)propionimidate, methyl-4-mercaptobutylimidate, methyl-3-mercaptopropionimidate, iminothiolene, o-carboxymethyl-hydroxylamine, azodiphenylpilmaleido, bis(sulfosuccinimidyl)sperate, 4,4'-diisothiocyano-2,2'-disulfonic acid stilbene, 4,4'-difluoro-3,3'-dinitrodiphenylsulfon, 1,5-difluoro-2,4-dinitrobenzene, p-phenylenediisothiocyanate, dimethyladipimidate, dimethylpimelimidate, dimethylsuberimidate, p-azidophenacylbromide, p-azidophenylglyoxal, N-hydroxysuccinimidyl-4-azidobenzoate, 4-fluoro-3-nitrophenylazide, methyl-4-azidobenzoimidate, N-5-azido-2-nitrobenzoyloxysuccinimide, N-succinimidyl-6-(4'-azido-2'-nitrophenylamino)hexanoate, 1,4-benzoquinone, N-succinimidyl-3-(2'-pyridyldithio)propionate, bisdiazobenzidine, hexamethylenediisocyanate, toluenediisocyanate, hexamethylenediisothiocyanate, N,N'-ethylenebismaleinimido, N,N'-polymethylenebisiodoacetoamide, and diazo compounds; or said condensation reagent is one or more compounds selected from the group consisting of carbodiimide derivatives represented by RN=C=NR (or R'), N-hydroxysuccinimide, tri-n-butylamine, butyl chloroformate, and isobutyl isocyanide.

48. A measuring chip for a surface plasmon resonance sensor comprising a metal layer, a plasma polymerization layer formed on said metal layer, a substance immobilized on the surface of said plasma polymerization layer, and an additional plasma polymerization layer or plasma-treated layer formed on said plasma polymerization layer.

49. A measuring chip according to claim 48, wherein said substance to be immobilized is selected from the group consisting of a nucleic acid, a non-immune protein, an immunoglobulin-binding protein, a sugar-binding protein, a sugar-recognizing sugar chain, a fatty acid or a fatty acid ester, a polypeptide or oligopeptide having ligand-binding activity, an immune protein, and an enzyme.

50. A measuring chip for a surface plasmon resonance sensor, comprising a metal layer, one or more plasma polymerization layers formed on said metal layer, and a substance immobilized on said plasma polymerization layer through a hydrophobic bond.

51. A measuring chip according to claim 50, wherein said substance to be immobilized is selected from the group

consisting of a nucleic acid, a non-immune protein, an immunoglobulin-binding protein, a sugar-binding protein, a sugar-recognizing sugar chain, a fatty acid or a fatty acid ester, a polypeptide or oligopeptide having ligand-binding activity, an immune protein, and an enzyme.

52. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a nucleic acid on the surface of said plasma polymerization layer.

53. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a non-immune protein on the surface of said plasma polymerization layer.

54. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing an immunoglobulin-binding protein on the surface of said plasma polymerization layer.

55. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a sugar-binding protein on the surface of said plasma polymerization layer.

56. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a sugar-recognizing sugar chain on the surface of said plasma polymerization layer.

57. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a fatty acid or fatty acid ester on the surface of said plasma polymerization layer.

58. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a polypeptide or oligopeptide having a ligand binding capability on the surface of said plasma polymerization layer.

59. A method according to any one of claims 52 to 58, wherein the plasma polymerization layer is formed by a plasma-treatment using a monomer material claimed in any one of claims 18 to 32.

60. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer by a plasma-treatment using a monomer material claimed in any one of claims 18 to 32, and then immobilizing an immune protein or enzyme on the surface of said plasma polymerization layer.

61. A measuring cell for a surface plasmon resonance sensor comprising a measuring chip according to any one of claims 1 to 51.

62. A measuring cell according to claim 61, wherein said chip is optically analyzed.

63. A surface plasmon resonance biosensor comprising a measuring chip according to any one of claims 1 to 51 or a measuring cell according to claim 61 or 62.

Claims

1. A measuring chip for a surface plasmon resonance sensor comprising (a) a metal layer and (b) one or more plasma polymerization layers formed on said metal layer.

2. The chip of claim 1 further comprising (c) a biologically active substance immobilized on the surface of said plasma

polymerization layer.

3. The chip of claim 2, wherein said biologically active substance is selected from nucleic acids, non-immune proteins, immunoglobulin-binding proteins, sugar-binding proteins, sugar-recognizing sugar chains, fatty acids or fatty acid esters, polypeptides or oligopeptides having ligand-binding activity, immune proteins, and enzymes.

4. The chip of claim 3, wherein

said nucleic acid is DNA, RNA or PNA,

said non-immune protein is either avidin, streptoavidin, biotin or a receptor,

said immunoglobulin-binding protein is protein A, protein G, or a rheumatoid factor,

said sugar-binding protein is lectin,

said fatty acid or fatty acid ester is either stearic acid, alachidic acid, ethyl stearate, ethyl arachidate, or ethyl behanate,

said polypeptide or oligopeptide is produced by using genetic engineering techniques or chemical synthesis methods, and

said immune protein is selected from antibodies, Fab fragments of antibodies and $F(ab')_2$ fragments of antibodies.

5. The chip of any of claims 1 to 4 further comprising an optically transparent substrate on which said metal layer is formed.

6. The chip of any of claims 1 to 5, wherein said plasma polymerization layer consists of two or more layers.

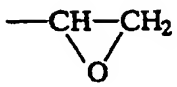
7. The chip of any of claims 1 to 6, wherein said plasma polymerization layer is formed of one or more monomer materials.

8. The chip of claim 7, wherein said monomer is selected from carbohydrate compounds comprising C and H, compounds containing nitrogen, compounds containing halogen, compounds containing sulfur, organic metal compounds, and organic silicon compounds; more particularly wherein said organic silicon compound is selected from tetramethylsilane, tetramethyldisiloxane, hexamethyldisiloxane, hexamethyldisilazane, hexamethylcyclotrisilazane, dimethylaminotrimethylsilane, trimethylvinylsilane, tetramethoxysilane, aminopropyltriethoxysilane, octadecyldiethoxymethylsilane, hexamethyldisilane, and divinyltetramethyldisiloxane.

9. The chip according to claim 8, wherein said compound containing nitrogen is CH_3-$(CH_2)_n$-NH_2 (wherein n is an integer from 1 to 6) and/or NH_2-$(CH_2)_n$-NH_2 (wherein n is an integer from 1 to 6), more particular wherein said compound containing nitrogen is selected form pyridine, ethylenediamine, hexamethylenediamine, n-propylamine, monoethylamine, triethylamine, diethylamine, allylamine, acrylamide, aniline, acryonitrile, 1,2,4-triazole, 5-amino-1H-tetrazole, propargylamine and acetonitile; and said compound containing sulfur is selected from dimethyl sulfide, methyl disulfide, ethanethiol, ethanedithiol, thiophen, mercaptoethanol and dithreitol.

10. The chip of any of claims 1 to 9, wherein plasma treatment is applied to said plasma polymerization layer with a polymeric or non-polymeric monomer; more particularly wherein said non-polymeric monomer material is selected from nitrogen, ammonium, hydrazine, hydrogensulfide, hydrogendisulfide, oxygen, hydrogen, water, halogen gas, and rare gas.

11. The chip of claim 7, wherein said monomer material comprises a compound having one or more groups selected from -COOH, -CHO, -SH, $-NH_2$, -OH, =NH, $-CONH_2$, -NCO, $-CH=CH_2$, =C=O and



12. The chip of claim 7, wherein said monomer material is a compound having ore or more -C=CCH_2OH groups.

13. The chip of any of claims 1 to 12, wherein said biologically active substance is immobilized on said plasma polymerization layer through a cross-linking reagent or a condensation reagent.

14. The chip of any of claims 1 to 13, comprising an additional plasma polymerization layer or plasma-treated layer formed on said biologically active substance.

15. The chip of any of claims 1 to 12, wherein said biologically active substance is immobilized on said plasma polymerization layer through a hydrophobic bond.

16. A method for producing a measuring chip for a surface plasmon resonance sensor comprising the steps of forming a metal layer on an optically transparent substrate, forming one or more plasma polymerization layers on said metal layer, and then immobilizing a biologically active substance on the surface of said plasma polymerization layer.

17. The method of claim 16, wherein said plasma polymerization layer and/or said biologically active substance are as defined in any of claims 3, 4 and 6 to 15.

18. A measuring cell for a surface plasmon resonance sensor comprising a measuring chip according to any of claims 1 to 15.

19. The measuring cell of claim 18, wherein said chip is optically analyzed.

20. A surface plasmon resonance biosensor comprising a measuring chip according to any of claims 1 to 15 or a measuring cell according to claim 18 or 19.

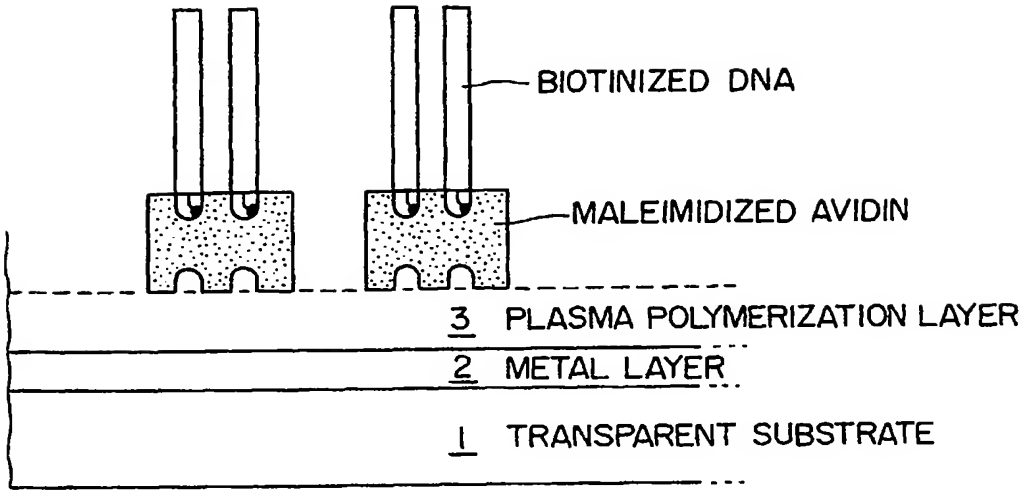

BIOTINIZED DNA
MALEIMIDIZED AVIDIN
3 PLASMA POLYMERIZATION LAYER
2 METAL LAYER
1 TRANSPARENT SUBSTRATE

FIG. 1

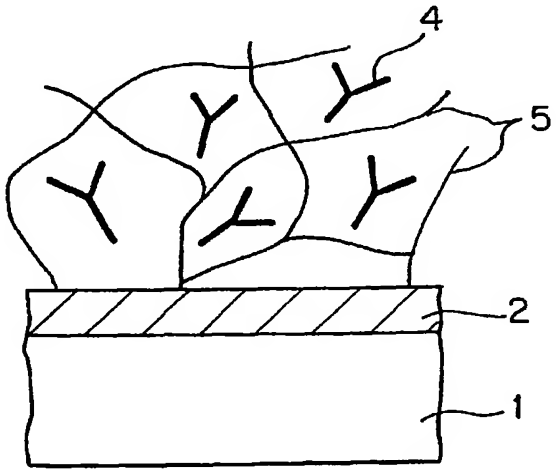

4
5
2
1

FIG. 2

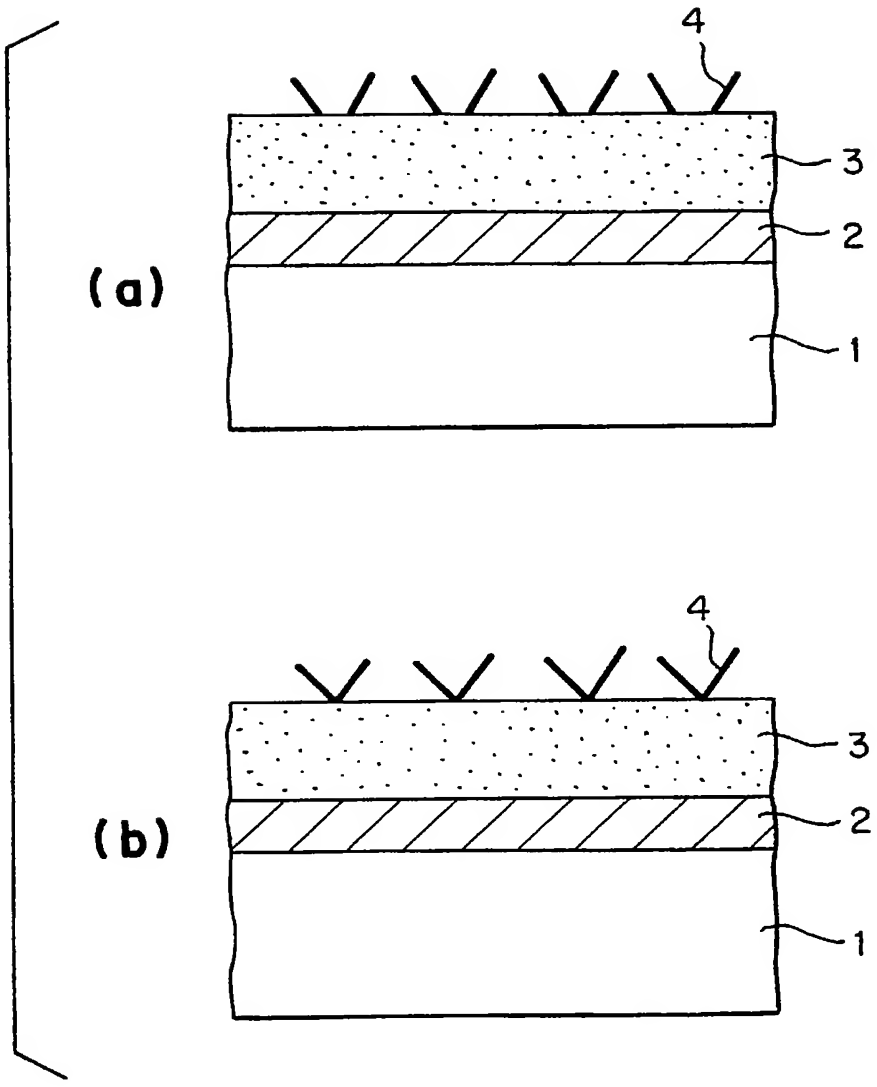


FIG. 3

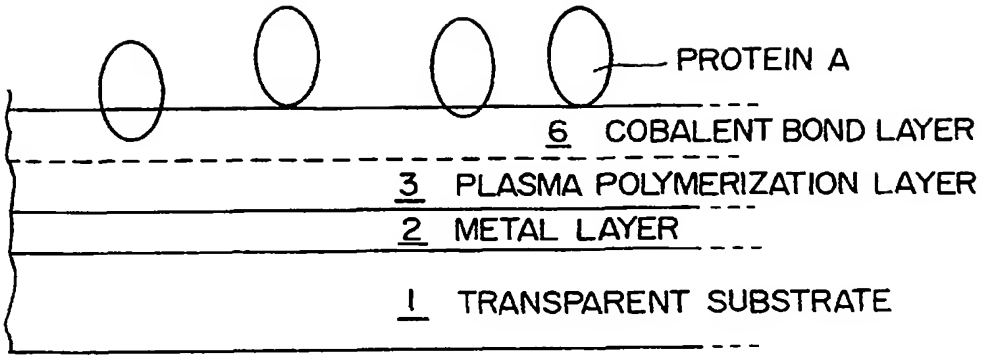

PROTEIN A
6 COBALENT BOND LAYER
3 PLASMA POLYMERIZATION LAYER
2 METAL LAYER
1 TRANSPARENT SUBSTRATE

FIG. 4

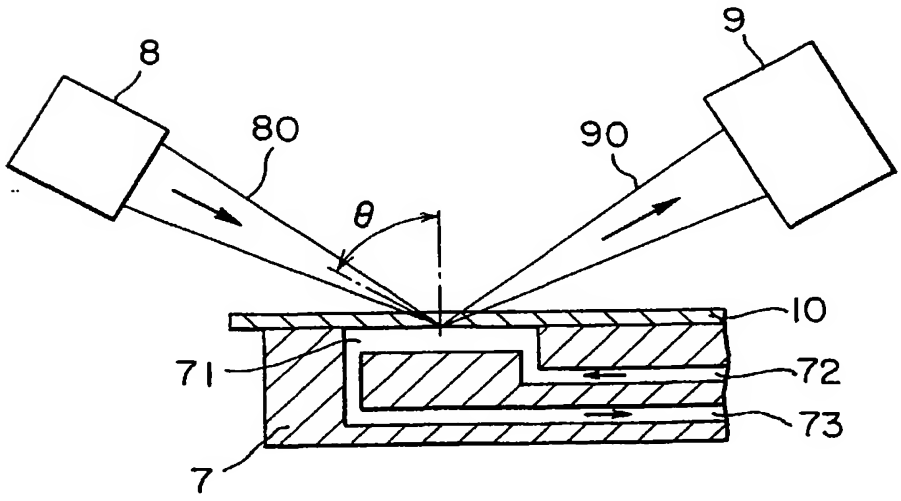

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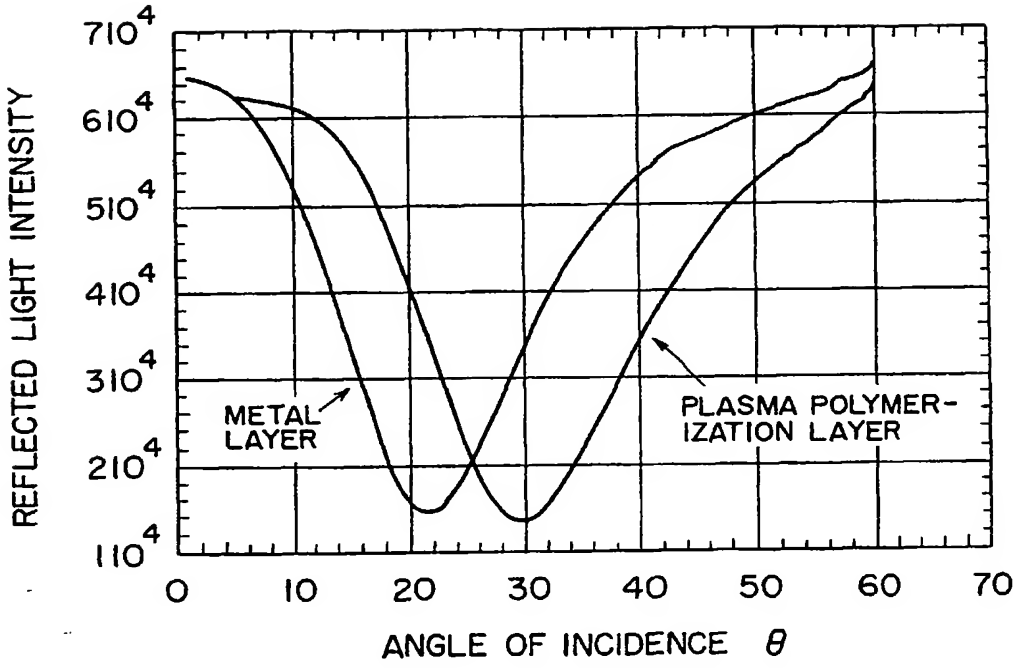
FIG. 5


REFLECTED LIGHT INTENSITY
710^4
610^4
510^4
410^4
310^4
210^4
110^4
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ANGLE OF INCIDENCE θ
METAL LAYER
PLASMA POLYMER-IZATION LAYER

FIG. 6

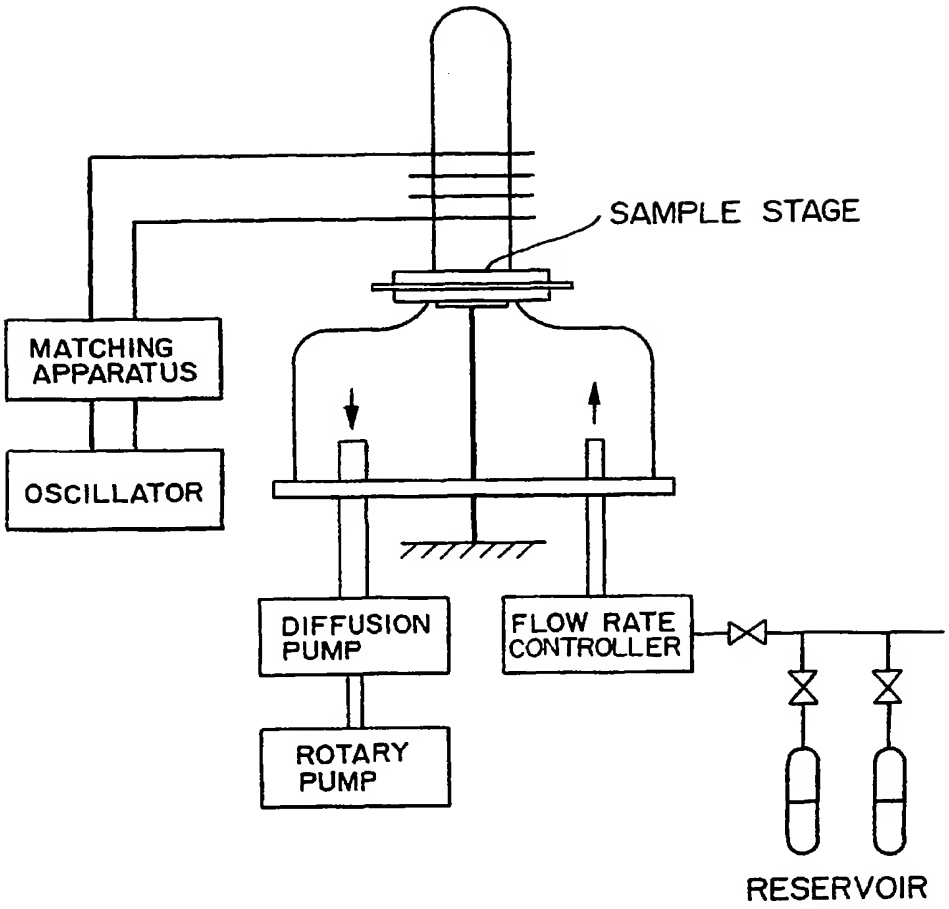
SAMPLE STAGE
MATCHING APPARATUS
OSCILLATOR
DIFFUSION PUMP
ROTARY PUMP
FLOW RATE CONTROLLER
RESERVOIR

FIG. 7

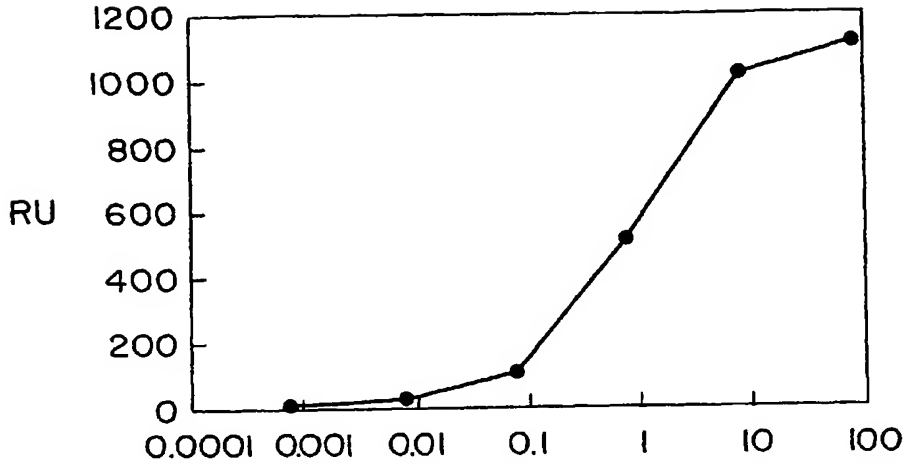
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FIG. 8

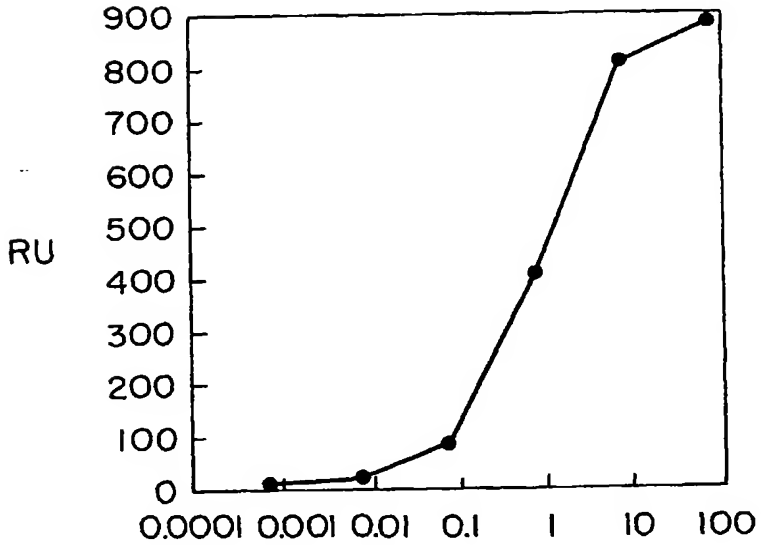
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FIG. 9

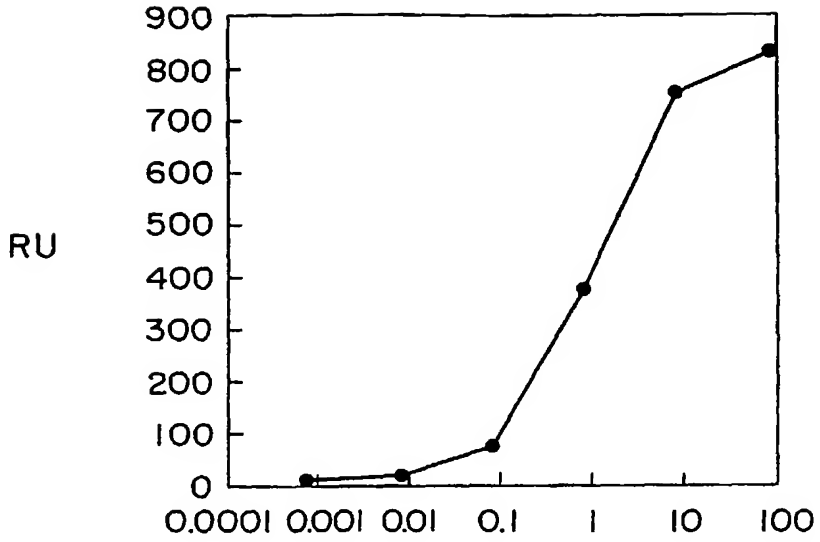
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FIG. 10

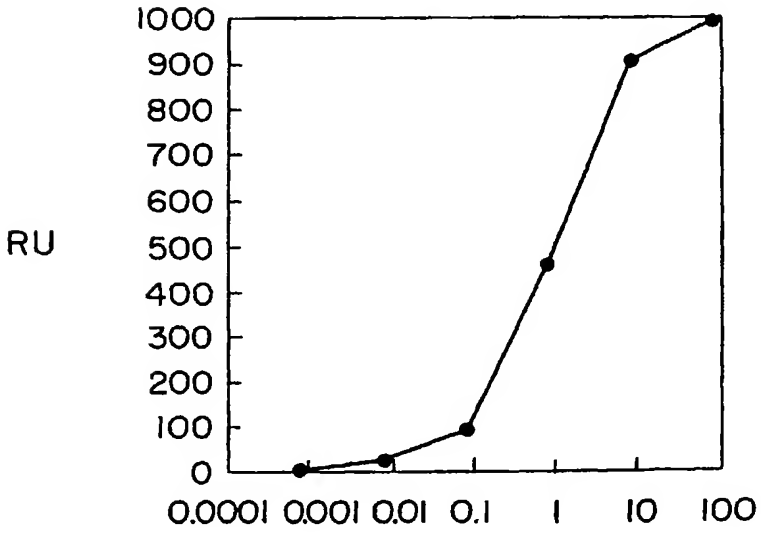
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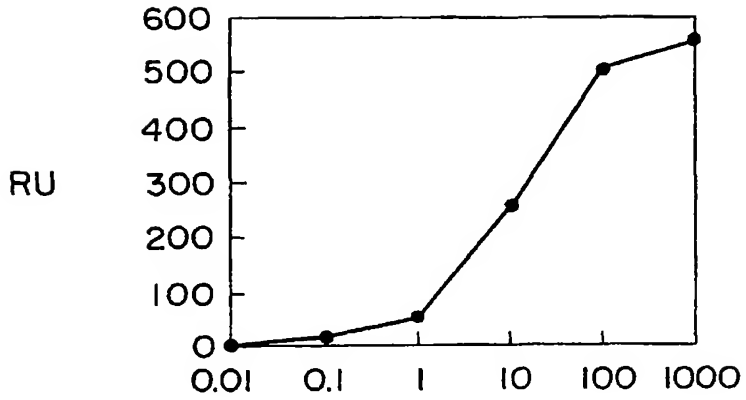
FIG. 11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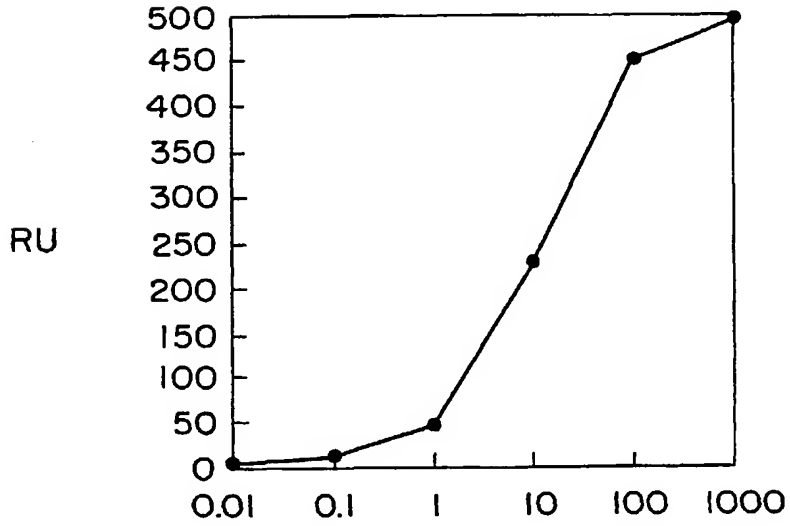
100
1000

FIG. 12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0
0.01
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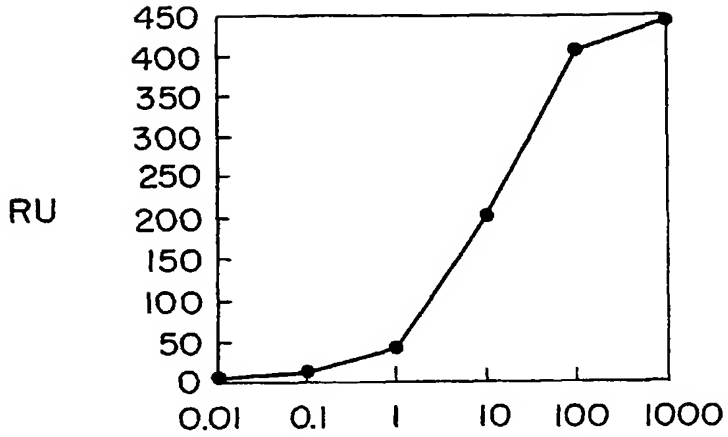
FIG. 13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0.1
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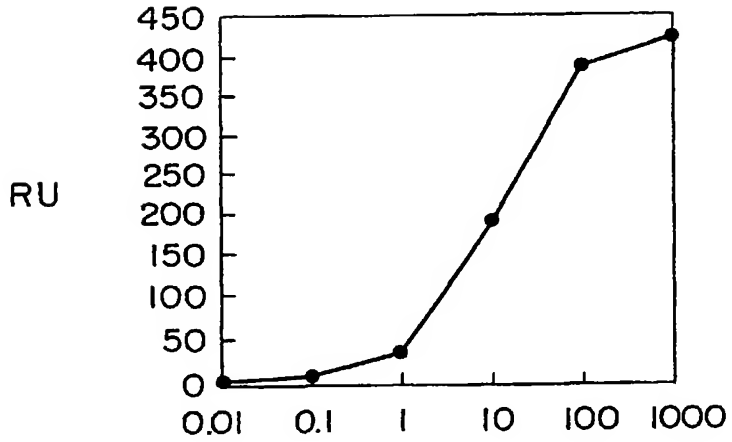
FIG. 14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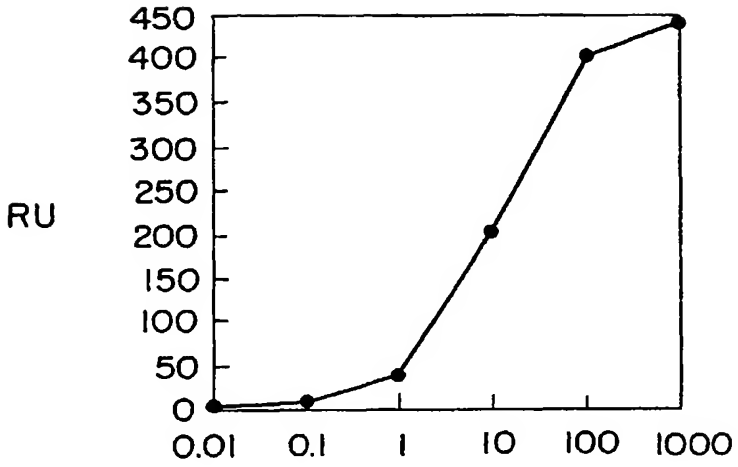
0.01
0.1
1
10
100
1000

FIG. 15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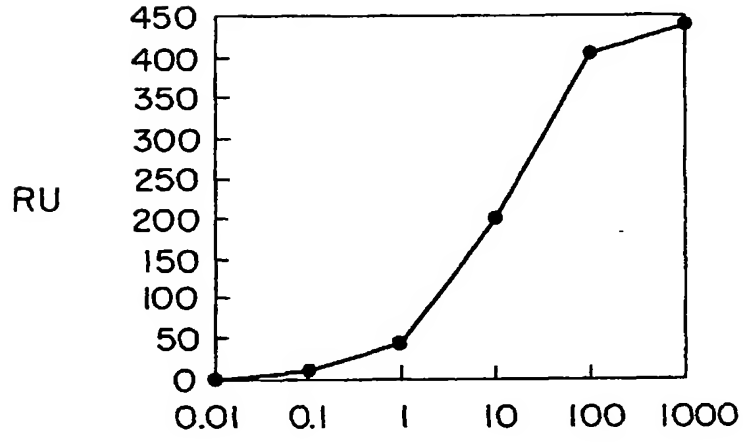
0
0.01
0.1
1
10
100
1000

FIG. 16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0
0.01
0.1
1
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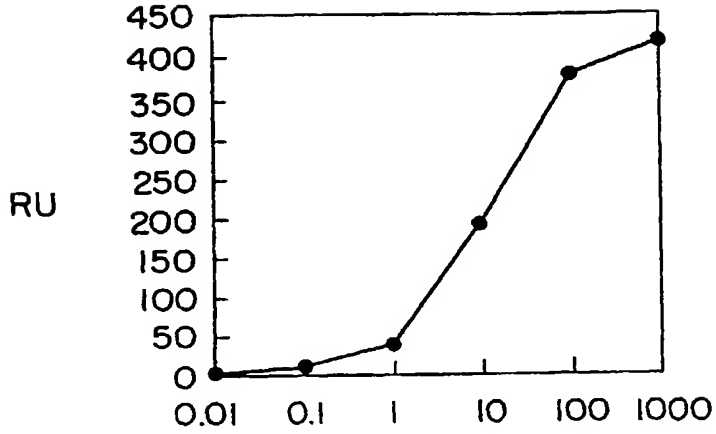
100
1000

FIG. 17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0.01
0.1
1
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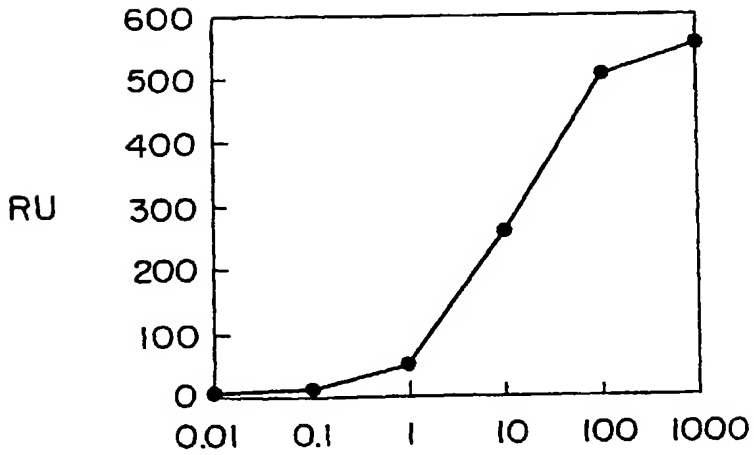
1000

FIG. 18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0.01
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FIG. 19

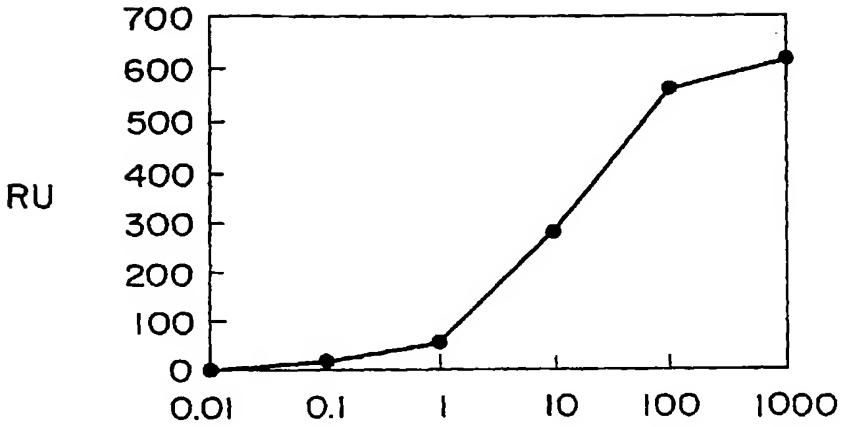
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FIG. 20

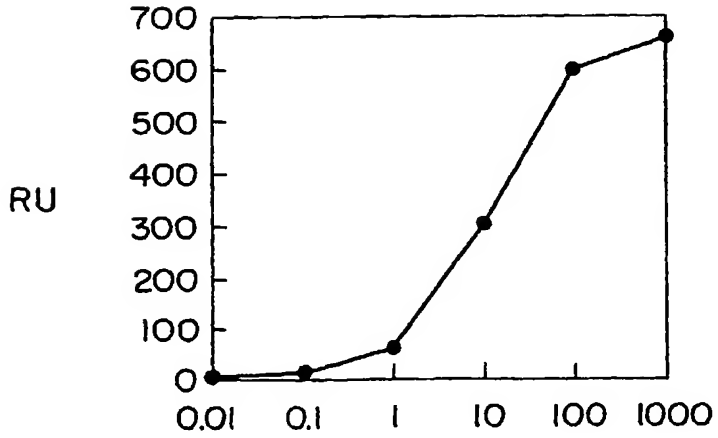
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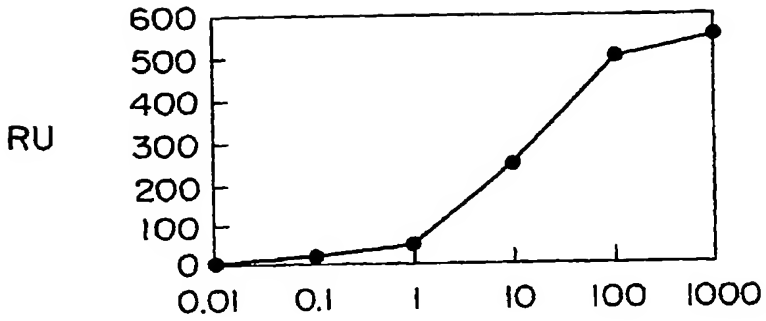
FIG. 21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0.1
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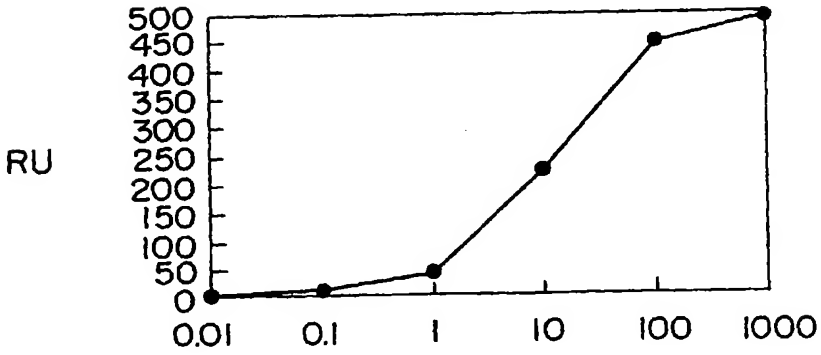
100
1000

FIG. 22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0.1
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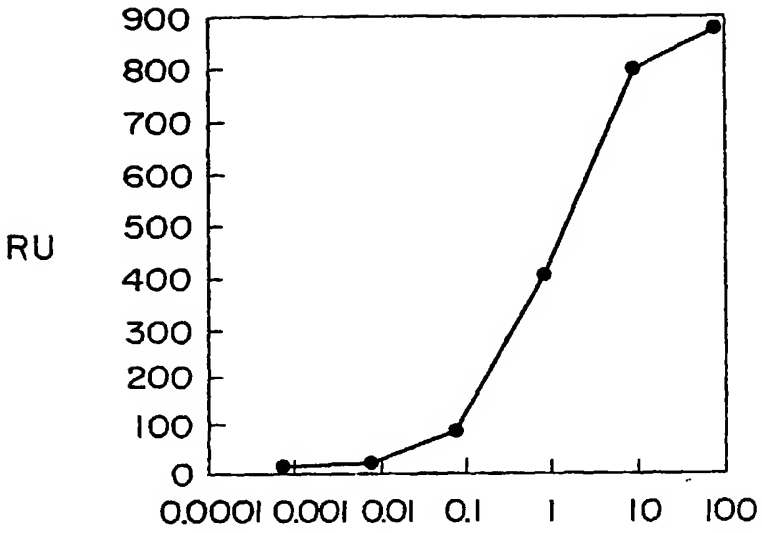
FIG. 23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0.0001
0.001
0.01
0.1
1
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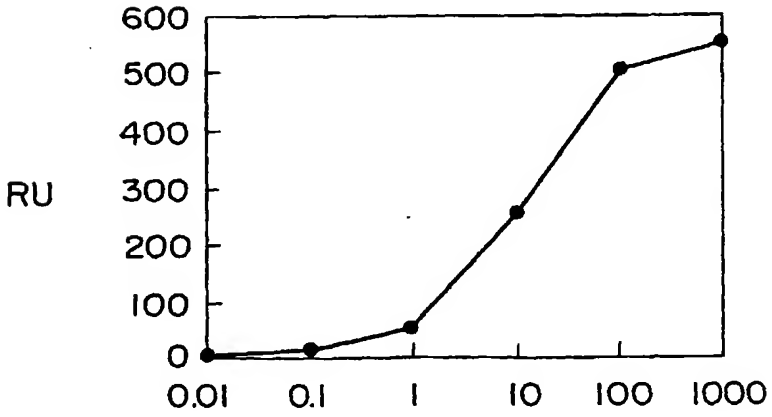
100

FIG. 24


RU
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0.01
0.1
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1000

FIG. 25

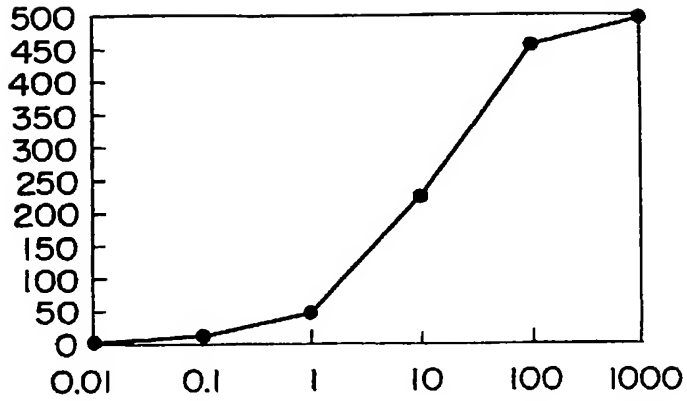

FIG. 26

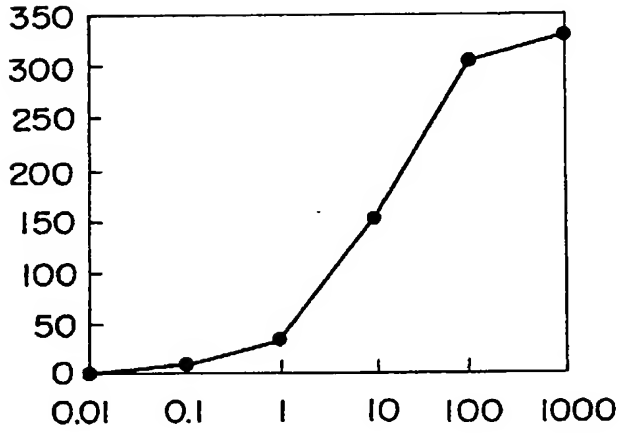

FIG. 27

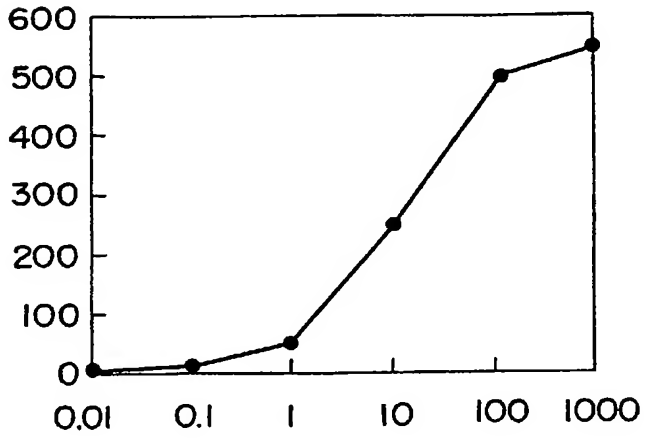

FIG. 28